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Improvement of lactic acid bacteria viability in acid conditions employing agroindustrial co-products as prebiotic on alginate ionotropic gel matrix co-encapsulation

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A B S T R A C T

Waste from foods processing are being widely studied due their content of many value added ingredients, like dietary fiber and bioactive compounds. The opportunities for recovery of components with a great economic potential from by-products are diverse. Apple marc derived from cider production and cactus pear peel flour, a popular consumed fresh fruit, besides inulin as control, were employed for co-encapsulation of probiotic thermotolerant lactic acid bacteria in alginate ionotropic gel matrix. Lactic acid bacteria viability, enhanced acid conditions resistant, where a higher microcapsule size was related to better viability and longer times resisting acid conditions. Inulin and apple marc resulted in higher microcapsules size (close to 100 μm) that could be employed to ensure the delivery of probiotic strains in colon throughout the gastro intestinal tract.

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1. Introduction

Food waste production covers the food life cycle from agriculture, transformation, retail and consumption, where in developed countries 39% of the losses occur in the transformation process. Food waste can be employed as raw material for new products and applications, since many residues have the potential to be reused in other production systems (e.g. biorefineries as a source of alternative energy, like ethanol). Besides, industrial symbiosis in recovering waste from food processing can be focus to obtain high added-value functional ingredients to be applied in the nutraceutical and pharmaceutical industry (Mirabella, Castellani, & Sala, 2014). Food industry faces the challenge to meet the increasing demand for food supplies, related to healthier products for a more balanced diet. Food processing is associated with the production of large waste or by-product streams, since not all parts of the plants can be consumed, like seeds and peels. In addition, since these parts have not been traditionally included in the diet, consumer acceptance is low and those parts are discarded. These by-products are an abundant source of biopolymers, such as proteins and carbohydrates, like dietary fiber, and bioactive components, such as carotenoids and phenolic compounds, and the opportunities for recovery of components from by-products are diverse with a great economic potential (van der Goot et al., 2016). Apple and Opuntia cactus are important part of agroindustrial productive chains. In Mexico, during 2015 the apple production reached 716.865 thousand tons. In Puebla region, 27.2 thousand tons of apple were produced during same period, destined mainly to cider production (around 240,000 l), generating an important amount of disposal as apple marc. Mexico is the main producer of cactus pear. Opuntia cactus pear production was around 352 thousand tons in 2015. Cactus pear is a cheap fruit with relatively high fiber content and hypoglycemic effect, but peel is not consumed (SAGARPA, 2016). Both apple marc and cactus pear peel are good alternatives to be employed as prebiotic ingredients.

Prebiotic is now defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" (Roberfroid, 2007). The effects on colonic flora and on the biochemistry and histology of the host bowel of prebiotics are associated with optimized colonic function and metabolism, increased fecal weight, a reduction in luminal colon pH, a decrease in nitrogenous end products and reductive enzymes, besides immune system modulation, supporting the logic of the

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use of prebiotics for promoting health benefits (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013).

The success of probiotics reflects the level of scientific evidence, besides the high consumer understanding and the demand for these health benefits. New innovate applications of probiotics are also emerging, like the recovery of skin immune functions in healthy subjects following UV exposure, the reduce crying time in colicky babies, possibly by improving gastric emptying, reduce anxiety-related symptoms in the general population stimulating of the parasympathetic nervous system, and a positive effect of on body weight in healthy overweight adults (Makinen, Berger, Bel-Rhlid, & Ananta, 2012).

Microencapsulation may be defined as a technology that involves the packaging of a gaseous, liquid or solid substance as the core or active phase within a secondary material like the matrix or encapsulant. Microencapsulation protects and stabilizes the encapsulated substance and allows its controlled delivery of the encapsulated substance at a desired site and rate when the capsule is exposed to specific conditions which provide a trigger or stimulus for the breakdown of the small capsule (Augustin & Sanguansri, 2017). In recent years, this process has been used as a useful tool for the stabilization of probiotic cells in functional food applications. Probiotic encapsulation protect cells against unfavorable environment, like processing, storage, subsequent consumption, gastrointestinal tract transit, to finally release them still viable and metabolically active in the intestine (Anal & Singh, 2007; De Vos, Faas, Spasojevic, & Sikkema, 2010; Ding & Shah, 2007). To employ alginate with another prebiotic material to encapsulate is referred as co-encapsulation (Dong et al., 2013). Co-encapsulation of probiotics and other important components like prebiotics protects food systems in the gastrointestinal tract much better, owing to synbiosis. Synbiotics are the mixture of probiotics and prebiotics to obtain a synergistic effect on human health (Bielecka, Biedrzycka, & Majkowska, 2002; Chen, Chen, Liu, Lin, & Chiu, 2005; Gibson & Roberfroid, 1995; Nazzaro, Orlando, Fratianni, & Coppola, 2012).

The objective of this work was to improve the viability of themotolerant lactic acid bacteria, reported as probiotic, employing agroindustrial co-products like cactus pear peel flour or apple marc flour, in alginate ionotropic gel matrix co-encapsulation, resulting in a symbiotic ingredient.

2. Materials and methods

Cactus pear (*Opuntia ficus* L.) peels were recovered from local fresh fruits establishments in Mexico City during the 2015 season (May to August). Marc from stripe apple (*Malus domestica*) was collected from Bodegas Delicia Zacatlán (Puebla, Mexico), an apple cider manufacture facility, during the 2015 production period (July to October). Cactus pear peels were collected weekly after fruit peeling and transported to University campus in plastic boxes, washed in cold tap water and stored under refrigeration (5 ± 1 °C) until processing. Peels were equilibrated at room temperature during 3 h before cut in small 2 × 2 cm pieces and dried at 60 °C during approximately 24 h in an air convection oven (Craft Instrumentos Científicos, México City). Apple marc was transported as well in plastic boxes and stored under refrigeration (5 ± 1 °C) until processing. Marc was equilibrated at room temperature during 4 h before dried at 60 °C during approximately 36 h in same convection oven. Dried samples were grounded in a grain mill and sieved consecutively in No. 100, 80, 50 and 20 sieves to obtain a regular and homogeneous powder named flour. Different collected lots were mixed to obtain a homogeneous single batch. Cactus pear peel and apple marc flours were stored in 1 kg hermetically dark containers until their use. Prebiotic potential of both cactus pear peel flour and apple marc flour had been previously reported (Diaz-Vela, Totosaus, Cruz-Guerrero, & Perez-Chabela, 2013; Pérez-Chabela et al., 2015).

Four strains of lactic acid bacteria, previously reported as probiotics with thermotolerant capacity (Ramirez-Chavarin, Wacher, Eslava-Campos, & Perez-Chabela, 2013), *Lactobacillus plantarum* UAM17, *Enterococcus faecium* UAM18, *Aerococcus viridans* UAM21b *and Pediococcus pentosaceus* UAM22a, were used. Lactic acid bacteria strains were reactivated in MRS broth at 37 °C for 24 h until obtain an optical density close to one (λ = 600 nm), equivalent to approximately 10^8 CFU/mL. The cell suspension was centrifuged in a Solbat centrifuge Model J40 (Solbat Instruments, Mexico City) at 2000*g* for 15 min to obtain a cellular pellet.

2.1. Bacteria microencapsulation

Alginate microcapsules were produced by emulsification internal ionotropic gelation of sodium alginate. Microencapsulation of bacteria strains was carried out adapting the technique of Homayouni, Reza-Ehsani, Azizi, Saeid-Yarmand, and Hadi-Razavi (2007). Cellular bottom of each strain was resuspended in 100 mL of Protanal SF 120 sodium alginate (FMC Biopolymers, Philadelphia) solution (2.5%, w/v), with 1% (w/v) of cactus pear peel flour or apple marc flour or Unicornio® agave inulin (Productos Naturales, México) as control, 200 mL of Mazola maize oil (ACH Food Co., Mexico) and 2.5 mL of Tween 80 (Sigma-Aldrich, St. Louis). The mixture was homogenized with magnetic stirring for 10 min at high speed (ca. 400 rpm). A mixture of 40 ml of maize oil with 2 ml of acetic acid was gently added to the emulsion during stirring in order to allow micro-beads formation. After 20 min, microcapsules were removed from the aqueous phase and washed twice with phosphate buffer solution pH 7.2. Microcapsules were stored in hermetically containers at 4 °C to allow fully harden during 24 h.

2.2. Particle size and encapsulation yield

The dimensions of the alginate capsule were determined using an objective micrometer on an optical microscope (Iroscope, Model BL-6 Mexico) at a 10× magnification, with a size range from 10 to 1000 μm.

To determine the encapsulation yield, the standard plate count method was used. Microcapsules (0.5 g) were dispersed in one mL of PBS (pH 7.0) and keeping at 100 rpm in a shaker during 30 min in order releasing cells from the microcapsules. The rehydrated samples were poured on MRS agar plates after appropriate dilutions in PBS solutions. Plates containing 20–350 colonies were counted after incubation at 37 °C for 24 h. The encapsulation yield was calculated as percent rate of the CFU/mL after encapsulation divided on the initial cell number (Rajam & Anandharamakrishnan, 2015).

2.3. Cell viability by flow cytometry

Cell viability was determined with a FACSCalibur flow cytometer (Becton Dickinson, San Jose). All parameters were collected and analyzed using Cell Questk® software (Becton Dickinson). L13152 LIVE/DEAD BacLight Bacterial viability kit (Molecular Probes, Eugene) was employed to determinate encapsulated cell viability. A 1:1 mixture of both dyes (SYTO 9, which stains intact cells in green, and propidium iodide that stains damaged cells in red) was prepared. 225 μL of this mixture was added to 225 μL of microcapsules dissolved in a PBS solution, as previously described, allowing to stain in the dark for 15 min at room temperature, according to the reported by Yáñez-Fernández, Ramos-Ramírez,

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